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A chemical and nutritional study of frozen haddock muscle

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INTRODUCTION

According to Pierce (52) the first important commercial use of haddock in the United States was about 1870 when smoked haddock fillets were first marketed as "finnan haddie" From that product the public readily turned to the use of fresh haddock, turning, in fact, with such enthusiasm that for the peak year, 1929, official statistics (79) show total landings of 261,653,000 pounds with a value of $9,142,000 to the fishermen of the United States alone. It is interesting to note that this increase in the use of haddock has paralleled very closely the development of modern refrigeration. In the late 19th century the individual fisherman attempted to rush his catch to some small seacoast town in order that he might dispose of it before spoilage set in. Now, by means of mechanical refrigeration, the time between the catching and consumption of the fish may be extended to several days, or by the even more modern methods of freezing, to several months. It has been a slow process, however, to educate the public to a point where it is willing to believe that a package of frozen fish, caught some months before, may be superior to that which a local merchant may have had in his showcase, on ice, for only a day or two. This erroneous view may have been strengthened by such statements as that of Vulte and Vanderbilt (83) that "Fish which has been frozen deteriorates rapidly when thawed and decomposition of a very undesirable nature sets in quickly. For this reason, fish should be eaten as fresh as possible." While this statement may be true in substance, it is necessary for us to consider with some care just how "Quickly" the decomposition sets in and perhaps study means of controlling it.

Since changes might take place which would affect the nutritive value of fish muscle without actual decomposition, a rather complete study of the subject would be necessary to absolutely prove the relative value, as human food, of fish muscle frozen by the various methods, either now in use or which might have commercial application. It was proposed, therefore, to make a rather complete chemical and nutritional study of the edible portion of the common haddock (Melanogramme aeglefinis) with especial emphasis on the commercial products as represented by the frozen fillets offered for sale. It was further proposed to study the chemical composition and nutritive value of various samples in an attempt to compare the relative value of whole fish frozen at sea as soon as caught by means of solid carbon dioxide, whole fish frozen by the Birdseye or so-called "quick" method after landing at the Boston Fish Pier but otherwise handled as the rest of the catch, and commercial fillets frozen by the Birdseye and Sharp (so-called "slow") methods.

By extending the work over the course of an year, it was hoped that some information would be gained concerning the seasonal variatim in the chemical composition of this species.

Chemically it was proposed to determine the moisture, ash, either extract, organic nitrogen, amino nitrogen, ammonia, sodium chloride soluble nitrogen fractions, copper, iron, manganese and phosphorus.

Biologically it was proposed to study the relative value of four types of frozen haddock muscle, as previously mentioned, as the sole source of protein in the diet of white rats as evidenced by growth, reproduction, and lacation. In addition assays were made for the vitamins A and D.

Review of Freezing Methods

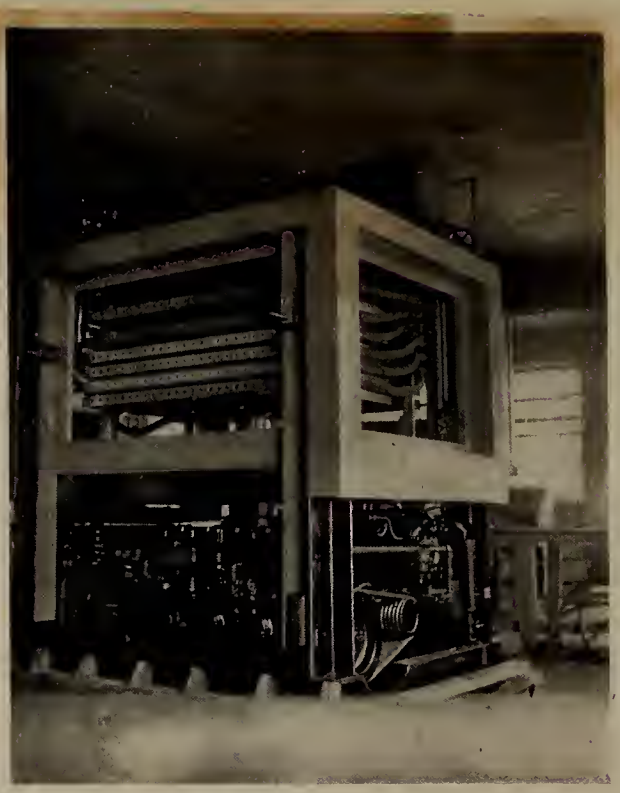
The commercial freezing of fish is far from a new idea. As pointed out by Birdseye and Fitzgerald (3) in a very complete review of the subject, Piper (53) as early as 1861 was experimenting with, in fact had patented a process for freezing fish in small cans surrounded with salt and ice. Since that time one method after another (71) (72) has been tried but none came into really extensive use until the advent of the two now-common methods known as the Birdseye or "Quick" and the Sharp or "Slow" methods.

The Sharp method consists essentially of packaging the products that are to be frozen and then storing them in a room at a temperature of approximately -35 degrees Fahrenheit. As will be readily understood in view of the poor conductance of heat by air, the freezing process is slow by this method. A five pound package of fish, for example, usually a package about ten by seven by two inches would take from eighteen to twenty-four hours to completely freeze. Not only is this an expensive loss of time when production is considered, but is considered by many to allow certain changes to take place in the structure of the product which may be undesirable.

The Birdseye method takes advantage of the rapid conductance of heat away from the packaged product by means of contact with either a metal belt, as in the case of the "Double-Belt Freezer" or a metal plate in the case of the "Multiplate Freezer." Pictures of these two types of modern freezers taken from an article by Poole (54) are shown in Figures #1 and #2.

AUTOMATIC FROSTER—TWO METAL BELTS GRIP THE PACKAGE CONTAINING ARTICLE TO BE FROZEN, AND CARRY IT THROUGH AN INSULATED TUNNEL IN WHICH CALCIUM CHLORIDE BRINE AT —45° F. IS SPRAYED ON THE TOP OF THE UPPER BELT AND THE BOTTOM OF THE LOWER ONE. AN AUTOMATIC DEVICE PREVENTS THE BRINE FROM REACHING THE PACKAGE.

Figure 1



BIRDSEYE PLATE FROSTER—AN APPARATUS WITH A NUMBER OF SUPERIMPOSED HOLLOW PLATES WHICH MAY BE OPENED TO RECEIVE THE PRODUCT TO BE FROZEN AND THEN CLOSED WITH ANY DESIRED DEGREE OF PRESSURE.

Figure 2

Lemon (26) (27) in writing for the Bureau of Fisheries Investigation - al Report #16 carefully described these two freezers as quoted below.

DOUBLE-BELT FREEZER

"The Double-Belt Freezer is constructed in units so that the capacity can be increased as the occasion arises. Each unit is composed of two corrosion-resisting metal belts placed one above the other, the length and width being the factors which determine the capacity of the machine. The upper belt is approximately 6 inches wider and is from 6 to 8 feet shorter than the lower. The tension of these belts is regulated by means of an adjustable spring and screw, and they are synchronized to move at the same speed in opposite directions. The motion is imparted to the belts by passing them over pulleys driven by an electric motor, the speed which can be regulated to coordinate with the time required to freeze the products being treated. These belts are almost entirely inclosed in an insulated cabinet or tunnel. The mechanical arrangement is such that the lower section of the upper belt and upper section of the lower belt move in unison in the same direction. The lower belt being longer protrudes at either end enabling the operator to place the material to be frozen in position with ease, as the belt moves toward the entrance to the tunnel.

"Upon entering the tunnel, contact is made with the lower section of the upper belt, thus forming a channel with closed top and bottom. Inside the insulated tunnel, calcium chloride brine at minus 50° F. is sprayed on the upper side of the lower section of the upper belt and lower side of the upper section of the lower belt. The regulation of pressure on the freezing product within the channel is accomplished by sets of rollers

working against the belts. The brine is prevented from running between the belts by the extended edges of the upper belt and by rollers which deflect the edges downward. The flexibility of the belt permits the freezing of several different size packages simultaneously. The rate of speed of the passage of thepackages can be controlled, thus making it possible to operate the machine continuously. When employing a brine solution at a temperature of minus 50° F. a package 2 inches thick is frozen in approximately 1½ hours. The capacity of this machine is estimated as being approximately 12,000 pounds of frozen product when operating on a basis of 24 hours per day.

"The solution employed as a refrigeration medium is chilled by means of direct expansion carbon dioxide coils in a tank of calcium chloride brine. The brine circulates continuously from the tank through the freezer and returns to the tank, completing the cycle. The compressors are of the 2-stage type, with a capacity of 10 tons each, and are driven by direct connected synchronous motors."

MULTIPLATE FREEZER

"The Multiplate Freezer is a modification of the Double Belt Freezer and is constructed in the form of a more or less portable unit. This freezer is composed of a series of plates, of varying dimensions, placed horizontally in an insulated cabinet. One of the most popular sizes for these plates is 48 by 52 inches surface and 2 inches thick. The plate is formed by casting aluminum alloy on a coil of ammonia pipe. Liquid ammonia is expanded directly in the coil, which makes it possible to reduce the temperature, in the plate, to 25° below zero F. The intake and discharge ends of the coils are connected into a header by means of a heavy

rubber hose. The distance between the plates is controlled by sets of pantographs, and the pressure of the plates upon the products to be frozen is regulated by a hydraulic cylinder under the bottom plate.

"The compressor and motor drive for machines of the larger capacities are placed outside of the unit. The capacity of the larger units is approximately 18,000 pounds of frozen products per 24 hours. The unit occupies 37.5 square feet of floor surface, and it is possible for several units to be operated from one compressor.

A small unit of this type, equipped with five plates, is entirely self-contained; the motor compressor is placed in a space underneath the insulated freezing chamber; the unit is supplied with castors which permit the shifting of the machine to different parts of the plant. The capacity of this size machine is 9,000 pounds of frozen products per 24 hours operation. It is possible to freeze packages ranging from $\frac{1}{2}$ inch to $3\frac{1}{2}$ inches in thickness. The temperature of this size machine is automatically controlled. The pressure of the plates upon the products to be frozen can be adjusted to suit the package, thus making it possible to freeze a variety of products. Another model of this apparatus is constructed with the plates independently movable, so that products varying in thickness from one half to eight inches may be frozen."

A very great advantage,,economically, of this latter type of freezer is the fact that it may be moved either by truck or train and used as a unit anywhere that running water and electricity are available. This, of course, not only saves the expense of transporting the raw materials from their source to a permanent freezing establishment, but makes it possible to freeze very perishable foods at their source while in the very best possible condition of freshness.

REVIEW OF LITERATURE

CHEMICAL

Among the earliest investigators to make a really thorough study of the chemical composition of fish was Atwater (2) who in 1888 published the results of several years work covering the analysis of fifty-two species of common American fishes, two species of European origin, and eleven species of American molluska and crustacea. In that work he determined the amount of water, ash, ether extract, and total nitrogen present. From these he calculated the food value using the familiar values of 4.1 Calories per gram for protein and carbohydrates and 9.3 Calories per gram for fat. In addition a limited amount of work was also done on the digestibility and retention of nutritive materials from the fish, using a man and a dog as subjects. Atwater's chemical analyses show considerable uniformity of composition, but since, unfortunately, no data are given in his report as to the season of the year during which the samples were caught, there is no way of explaining the variations, in the light of more recent work, as being, perhaps, the result of a normal seasonal variation.

Smith (65) in 1913 was among the early workers to study the effect of cold storage temperatures on the chemical composition of fish. In that year he compared the amounts of water, total solids, organic matter, ash, ammonia, and fat found in common flounder muscle when fresh and after storage periods of six and nine months. From his data one can only conclude that there was no significant change in any of the constituents for

which analyses were made.

Following somewhat the same line of work Clark and Almy (7) in 1920 studied the effect of cold storage on blue fish and white fish. They concluded that there was a slight increase in the amino acid and ammonia content of the muscle during storage, but a decrease in acid value and iodine number. In the light of changes to be discussed in this thesis under the question of "Ammonia as an Index of Decomposition" all of these changes may be interpreted to mean that there was a certain amount of decomposition during storage, due perhaps to an excessively high storage temperature.

In 1930 Reay (56) started a series of papers covering a rather extensive study of the effect of freezing on the protein of fish muscle. In the first of his papers, he concluded that the change may be largely the result of desiccation with the resulting change in the concentration of the salt solution of the cells, in turn, affecting the proteins.

The following year Lemon (25) published a paper based on attempts to prevent desiccation of frozen fish by coating them with a glaze of ice, cottonseed oil, corn oil, peanut oil, and hydrogenated cottonseed oil. All of these allowed the loss of moisture ranging from five and one half to twenty-three percent of the water present. These rather large losses may be partly explained by the fact that in the experiment he used a storage temperature of 15 degrees Fahrenheit as compared with the now-common holding temperature of -5 to -10 degrees Fahrenheit for frozen fish, fruits or meats.

In 1933 Reay (57) again attacked the problem from the standpoint of protein denaturation. He found that the most rapid change in the muscle could be expected between -2 and -4 degrees. His work covered temperatures

from 0 to -20 degrees Centigrade.

The following year Finn (15) published the results of similar studies on the juice extracted from fish muscle. He too found that at -2 degrees the denaturation of the proteins was very rapid with about twenty-four per cent of the total undergoing change in eighty-four days while at -20 degrees only three per cent was denatured in the same period of time. He too offered the question of the changing water content of the muscle at different temperatures as a possible explanation of the problem.

In 1935 similar studies were made by Reay (59) but with the idea of preserving fish in the best possible condition for smoke curing. This same work was again discussed by Lumley (31) in 1936.

The entire subject of fish freezing and the changes produced has been ably reviewed and discussed by several writers, especially Finn(14), Tressler (77), and Tressler and Evers (78). In this connection it should also be mentioned that a mimeographed bibliography, containing about one hundred and eighty references, dealing with the whole field of frozen foods, has been prepared for distribution by the Birdseye Frosted Foods Laboratory of Boston, Massachusetts.

In 1919 Greene (17) made a study of the variation in the fat and protein content of the King Salmon during the spawning migration, followed by a similar work by Davidson and Shostrom (10) on the Pink Salmon. Both of these were prompted by a desire to determine the optimum time and conditions for catching and canning these two valuable food fishes.

The question of seasonal variation in chemical composition of fish was mentioned again in 1936 by Reay (60) when he found the fat content of herring varying from about one to twenty-five per cent. This is also

undoubtedly closely associated with the feeding and migratory habits of this species.

The iron content of several species of fish has been studied and found to vary considerably by Peterson and Elvehjem (50) and Toscani and Reznikoff (75).

Peterson and Skinner (51) determined the amount of manganese in a number of fishes and crustacea but did not report any figures for haddock. Lindow and Peterson (29) analyzed halibut and bluefish for the same element with negative results.

Lindow, Elvehjem, and Peterson (28) determined the amount of copper in a number of foods including haddock, while Parks and Rose (39) examined about twenty species of fish for copper, iron, and manganese in an attempt to compare the composition of fresh and salt water fishes.

Among the rarer elements Bodansky (4) analyzed certain varieties of fish for zinc while Meulen (36) reported 0.03 milligrams of molybdenum per kilogram of whole fish.

Several workers have studied the ammonia content of fish muscle, usually from the standpoint of ammonia as a decomposition product of the nitrogenous material. These references will be dealt with in considerable detail in a later section of this thesis devoted to that question.

REVIEW OF LITERATURE

NUTRITIONAL

Few subjects could have been chosen for study on which there would have been less literature available than on the nutritive value of fish.

In 1888 Atwater (2) published, in connection with some chemical analyses, a limited amount of work based on the retention of fish by a man and a dog.

Osborne and his coworkers (43) (44) (45) (46) in 1908 and 1909 made rather extensive studies of the amino acids which could be identified in food products including scallops, halibut, chicken and ox muscle.

Not until 1918 did Drummond (11) publish his work in which he used the coagulable proteins from the muscle of cod, herring, and canned salmon in some feeding trials and reported that they had as high a biological value as similar products obtained from beef.

In 1934 Salgues (63) calculated the calorific value of about twenty Mediterranean fishes from their chemical composition.

Considerable work has been done on the protein value of certain meat products and some of these should be considered because of their relationship to this present work.

Hoagland and Snider (21) (22) showed that voluntary muscle, heart, liver, and kidney from cattle, sheep and hogs all have about the same nutritive value when fed in adequate amounts. They point out, however, that these seemed to be better utilized in thirty day test periods than when the work was extended to cover sixty days. This latter fact may be

of interest in considering some of the problems encountered in attempting to carry animals on a fish diet into the second and third generations.

Osborne and Mendel (48) likewise found ox muscle and hog liver to be good sources of protein for growth in rats but apparently did not go into the problem of reproduction and lactation.

MacLeod (32) found that when the amount of fresh meat in a rat diet was increased from 10 to 40 grams per week lactation was considerably increased. The work of Hitchcock (30) was similar in that it too showed that not only did female rats grow better but they raised larger and stronger litters when meat was added to a diet already considered to be adequate in proteins.

Nelson, Irwin, and Peet (38) found that a diet in which lean beef supplied all of the protein was adequate for growth and reproduction but failed in respect to lactation. Even increasing the amount of protein from 14.2 to 28.4 per cent and the feeding of additional yeast did not remedy the situation.

Daggs (9) working with dogs found that lactation varied with the source of protein. He reported that liver appeared to be better for the promotion of lactation than either round steak or egg.

Curtis, Hauge and Kraybill (8) working on various slaughter-house productsas sources of protein found cases of blindness among their rats which they attributed to a tryptophane deficiency.

Smith and Seegars (66) found that the growth of white rats was normal when liver was used to supply protein at a 20 per cent level. On a 15 per cent level, however, growth was subnormal and lactation was deficient in the first generation and lacking in the second. This fact is of interest because of the lactation problem met in this work, when a protein level of 19 per cent was supplied by frozen fish muscle.

Considerable work has been done on the vitamin content of fish oils, but that bearing most directly on this present work is by Pottinger, Lee, Tolle, and Harrison (55) on the liver oil of haddock. They reported that samples varied considerably but in general showed approximately fifty to seventy per cent of the vitamin potency of cod liver oil while in many cases the haddock liver oil exceeded the iodine number allowed under the U. S. P. specifications for cod liver oil.

DESCRIPTION AND PREPARATION OF SAMPLES

In this problem four series of samples of haddock were frozen at intervals of approximately one month, and extending over a period of eleven months. The four series of samples will be referred to in the following pages as Series B, Series C, Series D, and Series E.

All of the fish were caught by commercial trawlers of the General Sea Foods Corp. The fish designated as Series B were eviscerated as soon as caught, wrapped in parchment and immediately frozen by being placed in an insulated chest with solid carbon dioxide. This chest was so constructed and of such a size that it would hold four fifty-pound cakes of solid carbon dioxide. It was found that if the chest were not opened until about the last day of fishing, or about six or seven days after leaving port, about one third of the dry ice had evaporated. When the chest again arrived in port there was always enough of the solid carbon dioxide left to keep the fish solidly frozen. These fish were then transferred to one of the holding rooms at the Boston Fish Pier where they were kept frozen until they were shipped to the laboratory in solid carbon dioxide.

Series C consisted of fish commercially handled until reaching the Boston Fish Pier. That is, the fish were caught and eviscerated by the same means employed for Series B. They were packed in chipped ice in the hold of the vessel until unloaded at the pier. They were then dipped in a sodium chloride brine, wrapped in parchment, and frozen whole in a Birdseye multiplate freezer. They were stored in one of the holding rooms at the Boston Fish Pier until shipped to the laboratory at Amherst, packed in solid carbon dioxide.

Series D and Series E were commercial haddock fillets. Series D was prepared according to the procedure followed by the packers of the Birdseye Frosted Foods. These fish were filleted within two or three hours after unloading from the boat. The fillets were dipped in a 35° salimeter sodium chloride solution for about twenty-two seconds. After being wrapped in parchment the samples were packed in the regular commercial five-pound cartons and frozen in a Birdseye multiplate freezer. About thirty minutes were required for freezing before the cartons were packed in solid carbon dioxide for shipment to the laboratory.

Series E was prepared in the same way except for the method of freezing. The cartons were placed in a freezing room, according to the procedure of the Sharp method, at a temperature of about thirty degrees below zero, Fahrenheit. This required eighteen to twenty-four hours for complete freezing.

The season of the year that each sample was frozen will be designated in this paper by the use of exponents above the number of the sample. Ten samples of each series were frozen at intervals of a little over a month extending from August 20th, 1935 to July 10th, 1936. Thus, the four samples frozen on August 20th, 1935 will be spoken of as samples B^1, C^1, D^1, and E^1 respectively, while the samples frozen on September 20th, 1935 will be designated as samples B^2, C^2, D^2, and E^2. Following this system the samples frozen on July 10th, 1936 will be referred to as samples B^{10}, C^{10}, D^{10} and E^{10} respectively.

EXPERIMENTAL PART I

CHEMICAL STUDIES

WATER

In view of the amount of previous work done by Lemon (25) and Tressler (77) on the problem of the water content and the prevention of desiccation of frozen fish, any further study on that subject would have been superfluous. However the possibility of slight seasonal variation and the need of knowing accurately the water content of the samples in order to calculate the percentage composition of other constituents on both fresh and dry bases, it seemed advisable to make moisture determinations on representative samples of fish frozen by each of the four methods used in this work, and from each catch, thus covering a period of approximately one year.

As soon as the samples reached the laboratory, frozen in every case, representative samples of each, free from bones and skin, were taken and without defrosting were finely ground through a hand meat chopper, introduced into weighed weighing bottles and dried to constant weight in a vacuum oven at fifty-five degrees Centigrade. This temperature was used because, although it was high enough to give constant weight in from twenty-four to thirty-six hours, it was not high enough to perceptibly affect the small amount of oil in haddock muscle.

Following this procedure, results were obtained as shown in Table 1.

TABLE 1.

WATER CONTENT OF HADDOCK MUSCLE.

Date of Sample	Series B % H_2O	Series B Aver.	Series C % H_2O	Series C Aver.	Series D % H_2O	Series D Aver.	Series E % H_2O	Series E Aver.
Aug.20,35	80.76		81.19		81.00		80.46	
	80.78	80.77	81.23	81.21	80.92	80.96	80.33	80.40
Sept.20,35	80.44		81.63		80.32		80.31	
	80.75	80.60	81.65	81.64	80.46	80.39	80.38	80.35
Oct.24,35	79.26		80.47		80.73		80.84	
	79.29	79.28	80.01	80.24	80.56	80.65	80.38	80.61
Dec.4,35	-----		80.43		79.09		79.77	
	-----	-----	80.45	80.44	79.20	79.15	79.62	79.70
Dec.22,35	80.39		80.01		79.89		79.57	
	80.40	80.40	80.02	80.02	79.89	79.89	80.38	79.98
Feb.10,36	80.72		80.89		79.54		79.37	
	79.75	80.24	79.98	80.44	79.44	79.49	79.79	79.58
March12,36	80.61		79.94		80.69		80.65	
	81.06	80.84	79.80	79.87	80.52	80.61	80.59	80.62
April 10,36	80.04		81.24		81.69		79.80	
	80.17	80.11	81.78	81.51	81.52	81.61	79.74	79.77
May 6, 36	84.42		81.17		81.73		82.88	
	83.82	84.12	81.00	81.09	81.69	81.71	82.89	82.89
July 10,36	82.63		80.10		81.16		80.59	
	82.82	82.73	79.26	79.68	80.95	81.06	80.56	80.58
Averages	81.35		80.61		80.55		80.45	

After storage for nine and one half months, the moisture content of two samples (D and E samples from the catch of September 1935) was redetermined. It is interesting to note that in the case of the Birds-eye fillets (D) the moisture had decreased only 0.14 per cent while the Sharp fillets (E) during the same storage period had lost 0.96 per cent. Both of these samples had been glazed and wrapped according to the commercial practice and stored at a temperature of from five to ten degrees below zero, Fahrenheit.

A further study of Table 1, as a whole, will show that there was no significant difference in the water content of samples frozen by the four different methods nor any significant seasonal variation as found in the muscle of some species.

TOTAL ASH

As a means of determining the relative losses of soluble salts from the samples processed by the four methods previously described, it seemed advisable to determine the amount of total ash in all of the samples received. To do this, samples which had been vacuum dried at 55 degrees Centigrade in weighing bottles were weighed and introduced into small platinum dishes. The amount used was then calculated by difference. The samples were ashed to constant weight in an electric muffle oven set to maintain a very dull redness. The results of these determinations are given in Table 2.

One will notice at once that samples of Series B and Series C show considerably lower ash contents than do Series D and E. This can be readily explained in the light of the commercial practice of "brining" the samples before freezing. In the process the pieces of fish (whole in the cases of B and C and fillets in the cases of D and E) are lowered into a barrel of sodium chloride brine for about twenty-two seconds. This brine is made up to give a salimeter reading of 35°. Thus the amount of salt which penetrates the muscle is influenced considerably by the thickness of the piece "brined" and by any variation which may be allowed to take place either in the concentration of the brine or in the time the samples are allowed to remain in it.

In order to study the question of salt penetration, several commercial fillets from the same pack and frozen by the Birdseye method were subjected to the following analysis: Using a one half inch cork borer, "plugs" were cut out of each fillet. These were then cut into

TABLE 2

ASH CONTENT OF HADDOCK MUSCLE (DRY BASIS)

Date of Sample	Series B % Ash	Series B Aver.	Series C % Ash	Series C Aver.	Series D % Ash	Series D Aver.	Series E % Ash	Series E Aver.
Aug.20,35*	6.66		7.18		6.93		7.60	
	6.55	6.61	6.55	6.87	7.35	7.14	7.30	7.45
Sept.20,35*	6.24		6.32		9.33		10.64	
	6.44	6.34	6.21	6.27	9.18	9.26	9.97	10.31
Oct.24,35	5.99		6.89		7.13		6.96	
	6.01	6.00	6.87	6.88	7.25	7.19	7.12	7.04
Dec.4,35	----		5.94		8.10		8.43	
	----	----	5.93	5.94	7.99	8.05	8.39	8.41
Dec.22,35	6.24		5.81		6.97		6.81	
	6.28	6.26	5.69	5.75	6.99	6.98	6.85	6.83
Feb.10,36	6.32		6.30		8.14		8.15	
	6.24	6.28	6.36	6.33	7.93	8.04	7.84	8.00
Mar.12,36	6.49		6.28		7.53		7.69	
	6.40	6.45	6.18	6.23	7.44	7.49	7.53	7.61
Apr.10,36	6.30		5.88		8.53		8.64	
	6.29	6.30	5.88	5.88	8.43	8.48	8.56	8.60
May 6,36	7.01		7.23		8.47		8.66	
	7.08	7.05	7.16	7.20	8.57	8.52	8.61	8.64
July 10,36	7.25		6.64		7.60		7.24	
	7.34	7.30	6.49	6.57	7.09	7.35	6.87	7.06
Averages	6.51		6.39		7.85		7.99	

* Determinations made on fresh samples and calculated to dry basis. Other determinations on vacuum dried material.

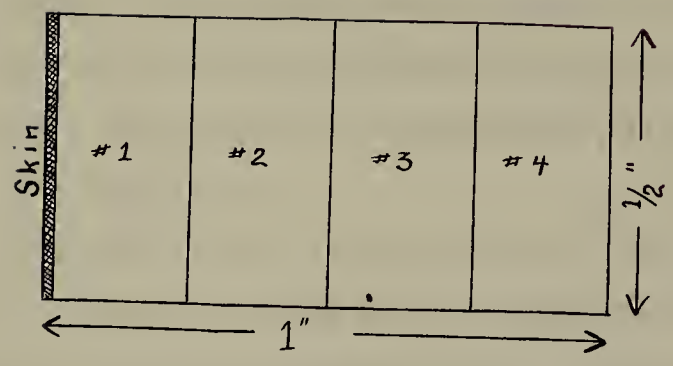


Figure 3

"Plug" cut from commercial fillet to show sodium chloride penetration.

four discs each as shown in Figure 3 in order to give one sample composed entirely of muscle located within about one quarter of an inch of the skin, a second, composed of muscle from one quarter to one half inch from the skin, a third sample one half to three quarters of an inch from the skin, and a fourth, a sample about a quarter of an inch thick but on the side of the fillet unprotected by skin. These four composite samples were then dried to constant weight at 55 degrees Centigrade in a vacuum oven. The dried samples were extracted with warm distilled water until free of chlorides. The four extracts were then made up to a known volume and aliquot portions analyzed for chlorides, using a modified Volhard-Arnold (82) method. The results are given in Table 3.

From Table 3 it can be seen that the penetration into the fish before freezing will depend upon the thickness of the samples, whether filleted or round, and whether protected by skin or not. This will explain, to a considerable degree, the greater amount of total ash found in the samples frozen after filleting than in those frozen "round". Since the thickness of the fillets will vary with the size of the fish an explanation is given for the variation in the total ash of the samples similarly processed but taken from different catches. Since the brining is not usually accurately timed, it is easy to believe that some samples may remain in the brine longer than others. Another source of variation may be found in the fact that a considerable number of fillets are brined in the same solution before it is discarded and a new one made. Due to the "drip" from the cut fillets some dilution of the brine will take place.

The examination of a few samples of freshly made brine and of samples taken after fish had been passing through them for about half

TABLE 3

Section	NaCl/g. Dry Sample
1	.0205
2	.0159
3	.0178
4	.0236
Average	.0195

an hour showed a loss of 15.5 per cent of the orignal salt present. That is, a representative brine sample, freshly mixed in the brine tank, gave a total ash of 9.84 per cent, while it showed a total ash content of only 8.32 per cent after being used for about half an hour.

ETHER EXTRACT

Following the method prescribed by the Association of Official Agricultural Chemists (41) fat, or more accurately, the ether extract was determined on all samples, not because it was anticipated that there would be any great difference in the samples processed by the four methods previously described, but because there has been some indication that there may be a seasonal variation in the amount of fatty material present in the muscle of certain fish. A survey of Table 4 will show that, although the haddock is very low in ether soluble material at all times, there is a slight rise during the late fall, which rapidly falls off during December and remains low until late in the summer.

TABLE 4

ETHER EXTRACT (DRY BASIS)

Date of Sample	Series B % Fat	Series B Aver.	Series C % Fat	Series C Aver.	Series D % Fat	Series D Aver.	Series E % Fat	Series E Aver.
Aug.20,35	.86		.61		.59		.74	
	.64	.75	.65	.63	.65	.62	.80	.77
Sept.20,35	1.35		.99		.77		.63	
	1.34	1.345	.88	.935	.77	.77	.62	.62
Oct.24,35	.72		.64		.53		.64	
	.70	.71	.80	.72	.53	.53	.71	.675
Dec.4,35	*		.45		.65		.60	
			.49	.47	.63	.64	.58	.59
Dec.22,35	.39		.39		.48		.53	
	.45	.42	.48	.435	.51	.495	.51	.52
Feb.10,36	.58		.52		.60		.54	
	.52	.55	.51	.515	.61	.605	.63	.585
March 12,36	.54		.54		.59		.57	
	.58	.56	.56	.55	.59	.59	.61	.59
Apr.10,36	.63		.55		.55		.69	
	.66	.63	.53	.54	.60	.575	.67	.675
May 6,1936	.52		.56		.51		.51	
	.58	.55	.62	.59	.49	.50	.48	.49
July 10,36	.44		.55		.57		.64	
	.43	.435	.55	.55	.59	.58	.62	.63
Averages	.661		.594		.591		.615	

* Samples lost at sea.

TOTAL ORGANIC NITROGEN

Recognizing the fact that fish muscle is a valuable food, primarily because of its high protein content, it seemed important to determine whether the common methods of freezing altered the amount of this constituent to an appreciable extent. With this end in view, the total organic nitrogen of all samples was determined, following the method prescribed by the Association of Official Agricultural Chemists (42) for meat products. In this work the samples were ground in a hand meat chopper and dried in a current of warm air (about 55 °C.) , then ground to a fine powder in a mortar and dried to constant weight at 55 degrees in vacuo in weighed weighing bottles. Weighed samples were then transfered to Kjeldahl flasks, digested using metallic mercury as a catalyst, and distilled into 0.5 normal sulphuric acid.

The results of these analyses are given in Table 5. It will be noted that there was a striking uniformity not only among the samples frozen by the various methods but also among the samples taken throughout the year. Considerable difficulty was experienced in obtaining perfectly uniform samples free of very small bones. This is believed to account for any variations noted. To overcome this difficulty as completely as possible, after the first two samples (Aug. 20th and Sept. 20th, 1935) very large samples were dried, very finely ground, remixed, sampled, and dried in vacuo before being analyzed.

TABLE 5

ORGANIC NITROGEN (DRY BASIS)

Date of Sample	Series B % N.	Series B Aver.	Series C % N.	Series C Aver.	Series D % N.	Series D Aver.	Series E % N.	Series E Aver.
Aug. 20,35	16.59		16.18		17.23		16.84	
	16.69	16.64	16.65	16.42	15.49	16.36	16.43	16.64
Sept.20,35	16.13		15.74		15.91		14.40	
	16.03	16.08	16.23	15.99	15.45	15.68	14.10	14.25
Oct.24,35	15.22		15.09		15.06		15.05	
	15.24	15.23	14.98	15.04	15.06	15.06	15.10	15.08
Dec.4,35	*		15.41		14.93		14.91	
			15.54	15.48	14.90	14.92	14.91	14.91
Dec. 22,35	15.55		15.49		15.09		15.11	
	15.44	15.50	15.51	15.50	15.19	15.14	15.07	15.09
Feb 10,36	15.65		15.59		15.13		15.23	
	15.65	15.65	15.56	15.58	15.14	15.14	15.20	15.22
March 12,36	15.56		15.62		15.38		15.37	
	15.53	15.55	15.58	15.60	15.38	15.38	15.38	15.38
April 10,36	15.70		15.71		15.08		14.93	
	15.55	15.63	15.56	15.59	15.10	15.09	15.04	14.99
May 6,36	14.99		15.12		14.71		14.80	
	15.09	15.04	15.14	15.13	14.71	14.71	14.81	14.81
July 10,36	15.16		-----		14.16		14.40	
	15.14	15.15	15.04	15.04	14.13	14.15	14.48	14.44
Agerages	15.61		15.56		15.16		15.08	

*Sample lost at sea.

AMMONIA.

As a result of the extensive work of Lucke and Geidel (50), Tillmans and Otto (74) and Tillmans, Hirsch and Kuhn (73), it is generally accepted that the ammonia content of fish or meat bears a rather definite relation to the state of preservation of the nitrogenous material of those products. Thus, since in this study we are interested in the relative merits of certain methods of freezing fish muscle for human consumption, it seemed advisable to determine the amount of ammonia in all samples as a means of checking the quality of the fish frozen by the four methods previously outlined.

Using a modified Folin aeration method (40) discussed in greater detail later (page 34), results were obtained as shown in Table 6.

It will be noted that there was practically no free ammonia present in any of the samples, with a maximum of .26 milligrams per gram of fresh muscle in only one sample. This one sample, however, had almost twice as much ammonia as any other similar sample, thus rather definitely indicating that somewhere in processing or storage, one sample had not retained its freshness as completely as had the others.

TABLE 6

AMMONIA

(Milligrams ammonia per 100 Grams Fresh Muscle.)

Date of Sample	Series B Ammonia	Series B Aver.	Series C Ammonia	Series C Aver.	Series D Ammonia	Series D Aver.	Series E Ammonia	Series E Aver.
Aug.20,35	0.00		0.10		0.00		0.10	
	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Sept.20,35	0.10		0.00		0.15		0.136	
	0.10	0.10	0.15	0.075	0.132	0.141	0.153	0.145
Oct.24,35	0.10		0.17		0.10		0.26	
	0.10	0.10	0.10	0.135	0.10	0.10	0.13	0.20
Dec.4,35	*		0.07		0.09		0.10	
			0.085	0.078	0.085	0.088	0.10	0.10
Dec.22,35	0.08		0.11		0.09		0.08	
	0.10	0.09	0.14	0.125	0.08	0.085	0.09	0.085
Feb.10,36	0.09		0.09		0.09		0.12	
	0.09	0.09	0.09	0.09	0.10	0.095	0.15	0.135
March 12,36	0.00		0.09		0.07		0.07	
	0.03	0.015	0.00	0.045	0.00	0.035	0.00	0.035
April 10,36	0.08		0.14		0.13		0.14	
	0.08	0.08	0.14	0.14	0.11	0.12	0.12	0.13
May 6,36	0.037		0.065		0.055		0.055	
	0.055	0.046	0.082	0.074	0.073	0.064	0.073	0.064
July 10, 36	0.051		0.068		0.068		0.068	
	0.060	0.056	0.085	0.077	0.102	0.085	0.102	0.085
Averages	0.064		0.089		0.080		0.102	

*Samples lost at sea.

THE AMMONIA CONTENT OF HADDOCK MUSCLE AS AN INDEX OF THE STATE OF PRESERVATION. *

At this point it seemed fitting to digress a little from the originally planned work in order to study the rate of change in the ammonia content of the fish muscle in relation to its state of preservation, and to compare the rates of change at various temperatures in the four samples studied.

As Reay (58) has pointed out, there are three major methods available for estimating the state of preservation of fish muscle; (a) The Organoleptic Method, (b) Bacteriological Methods, and (c) Chemical Methods. The fish merchant or consumer must depend largely on the first method. For more accurate study, one of the other two must be used. In this connection we have the work of Stewart (69) in which the rate of bacterial multiplication was followed, Tressler (76) studied the relationship between amino acid content and the condition of fish muscle while Almy (1) followed the decomposition of fish flesh by means of the hydrogen sulphide formed. Tillmans and Otto (74) and Lücke and Geidel (30) approached the same subject form the standpoint of ammonia nitrogen liberated.

* The subject matter of this section was incorporated into a paper which was read before the Biological Section of the American Chemical Society at their semi-annual meeting at Chapel Hill, N.C. on April 13, 1936.

More recently we have the work of Stansby and Lemon (67) based on an electrometric titration to determine the buffering effect of the protein molecule before and during decomposition. In 1935 Stansby (68) published an additional method for testing the quality of fish tissue, based on the condition of the oil which it contained.

In this present work an attempt has been made: (1) to follow the decomposition of the protein of fish muscle by means of the ammonia nitrogen formed; (2) to compare the rates of decomposition of fish proteins, fresh and after having been frozen by various methods and defrosted.

Experimental.

The Rate of Decomposition of Fish Muscle at Various Temperatures.

During the course of an extended study of the chemical composition of the muscle of the common haddock (Melanogramme aeglefinis) it was noticed that there was a striking uniformity in the ammonia nitrogen present in fresh samples. On one occassion, however, a sample showed an abnormally high quantity. Investigation showed that, due to electrical failure, the refrigerator in which the samples were kept, had not functioned properly and for several hours had approached the temperature of the laboratory on a warm summer evening. From a number of preliminary analyses, made on samples which had been held at various temperatures, it was evident that the temperature had materially affected the rate of decomposition. In order to more accurately compare the rates of de-

composition at various, but controlled temperatures, the following experiment was conducted.

A sample of fish muscle, free of bones and skin, was finely ground in a meat chopper, mixed, and divided into four parts. Each part was stored in a glass stoppered bottle at a different temperature as follows: (A) held in a frozen condition in the freezing unit of a modern household electric refrigerator, (B) held in the food compartment of the same refrigerator set to maintain a temperature of 4-5° C., (C) held in the food compartment of an ordinary ice refrigerator which was found to maintain a temperature of about 9-10° C., and (D) held in a water bath at 24-5° C. to simulate summer conditions. Immediately after grinding and then at regular intervals, as indicated in Table 7 and in Figure 4, the ammonia was determined for a sample from each bottle.

The method used was essentially that outlined by the Association of Official Agricultural Chemists for the determination of ammonia in meat and meat products. (40) A ten gram sample of the finely ground material was placed in a large test tube and thoroughly mixed with 10-15 ml. of ammonia free distilled water, using a heavy glass rod. To this were then added 1.0 ml. of a saturated solution of potassium oxalate and 20 ml. of a saturated solution of sodium carbonate. This was then rapidly aerated for four and one half hours. The ammonia was caught in 0.5 normal sulphuric acid and finally the excess acid was titrated with 0.1 normal sodium hydroxide. It will be noted that in the case of sample D decomposition had progressed so far in forty-eight hours that the sample had an objectionable odor, while the ammonia had increased

TABLE 7

Days held after Grinding.	Milligrams NH_3 per 100 Grams of Sample (A)	(B)	(C)	(D)
0	8.5	6.8	8.5	6.8
2	8.5	6.8	7.7	89.3 (1)
4	3.4	5.0	---	231.2
7	5.1	6.0	127.5(2)	324.7 (3)
9	6.8	7.7	161.5	
14	4.3	26.4		
21	---	89.2 (1) (4)		
28	7.7	82.5		

(A) Ground haddock stored at 0° C.

(B) Ground haddock stored at 4-5° C.

(C) Ground haddock stored at 9-10° C.

(D) Ground haddock stored at 24-25° C.

(1) Objectionable odor of decomposition.

(2) Very objectionable odor of decomposition.

(3) Decomposition progressed to such an extent that sample discarded after analysis.

(4) Pronounced "fishey" odor in addition to decomposition.

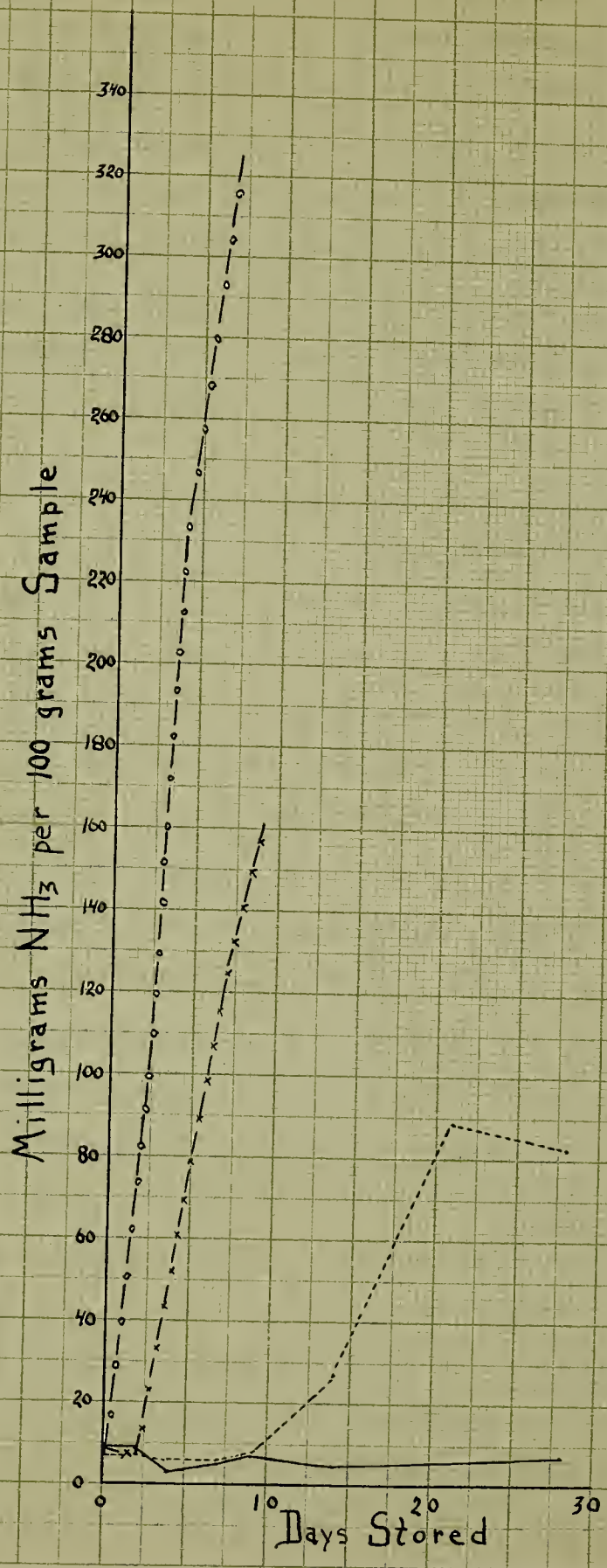


Figure 4

Storage Temperature

0° C.

4-5° C.

9-10° C.

24-5° C.

to 89.3 milligrams per 100 grams of sample. By the seventh day this sample had decomposed to such an extent that it showed an ammonia content of 324.7 milligrams per 100 grams of sample. On the other hand, sample A, held in a frozen condition, showed no signs of decomposition either in respect to ammonia content or general appearance. A similar sample, held for a period of several months in a frozen condition, likewise showed no increase in ammonia beyond that of experimental error.

The Rate of Decomposition of Fresh and Defrosted Samples.

Although previous analyses indicated that there was no great change in the fish proteins due to freezing, the following experiment was conducted in order to more carefully compare the rate of decomposition of samples handled according to commercial practice under identical conditions of storage. Four samples were compared: E, Fresh Haddock fillets purchased locally from a reputable market. The fish were displayed round, in an electrically refrigerated showcase and filleted as the trade demanded. F, Commercial fillets, frozen by the slow or Sharp method. G, Commercial fillets frozen by the quick or Birdseye method. H, Fish frozen round by the Birdseye method but otherwise handled in the same manner as the commercial fillets, that is, caught by trawl, gutted at sea as soon as caught, and brought to land stored in bins with crushed ice in the hold of the vessel. The fish used in E, were claimed by the manager of the store in which purchased to have arrived that same morning, in ice, by motor truck from Boston about ninety miles away. The ammonia content of this sample compared very closely with other commercial samples purchased locally. Samples F, G, and H. after being landed at the Boston Fish Pier, in

TABLE 8

Days held after Grinding.	Milligrams NH_3 per 100 Grams of Sample. (E)	(F)	(G)	(H)
0	8.5	8.5	8.5	6.8
1	8.5	---	---	---
3	---	13.6	12.8	---
7	59.5 (1)	29.8	----	6.0
9	----	89.3 (3)	73.1 (3)	7.7
11	83.3 (2)	----	----	---
14	----	188.7	167.5	26.4
21	139.4 (4)	191.7 (4)	183.7 (4)	89.2

(E) Fresh haddock fillets purchased locally

(F) Commercial haddock fillets frozen by Sharp method

(G) Commercial haddock fillets frozen by Birdseye method

(H) Fish frozen round by Birdseye method.

(1) "Fishey" odor.

(2) Objectionable odor of decomposition.

(3) Stale odor, distinctly different from putrifactive odor.

(4) Decomposition progressed to such an extent that sample discarded after analysis.

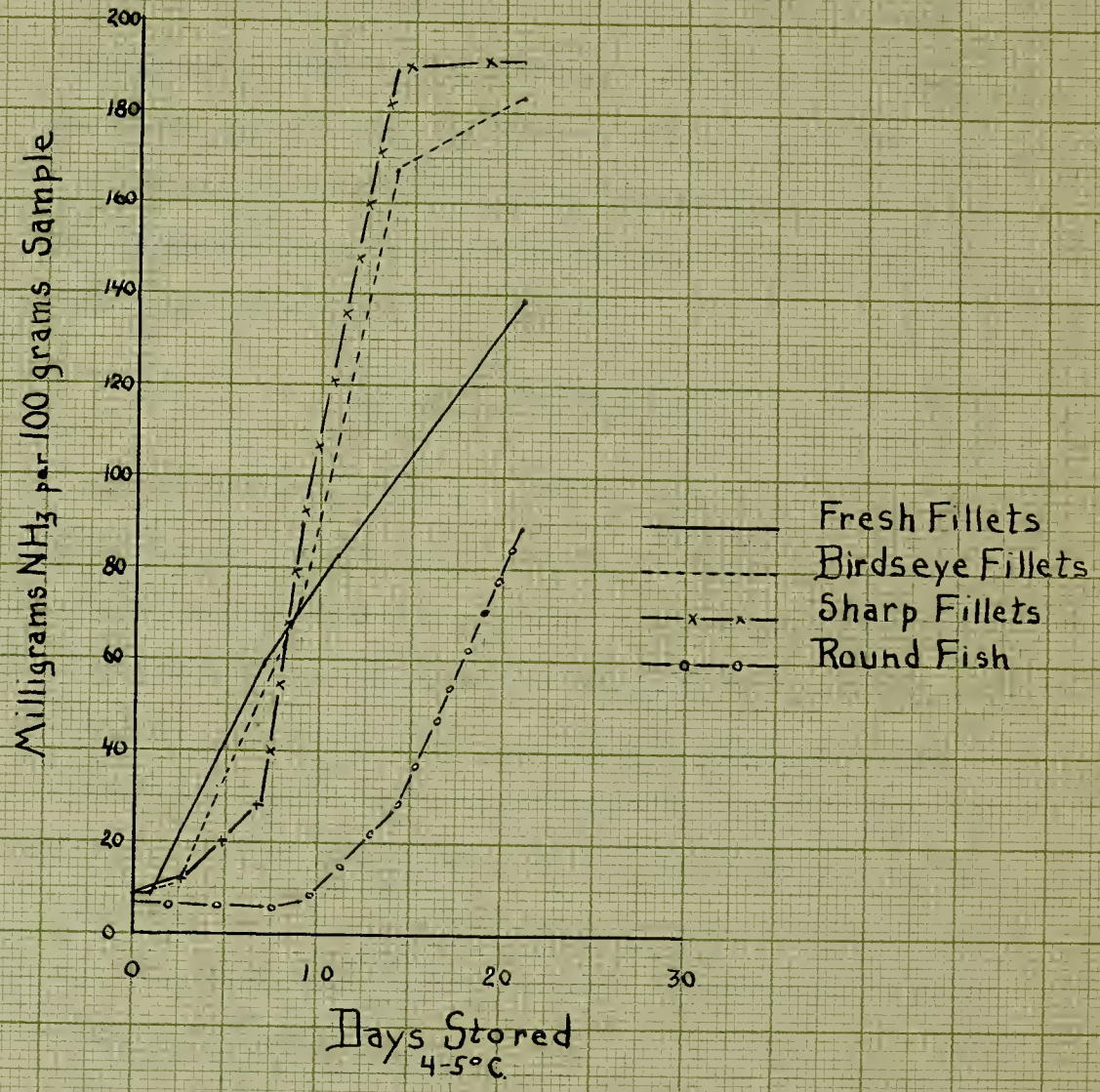

Milligrams NH_3 per 100 grams Sample
200
180
160
140
120
100
80
60
40
20
0
0
10
20
30
Days Stored
4-5°C.
Fresh Fillets
Birdseye Fillets
Sharp Fillets
Round Fish

Figure 5

ice, had been frozen by the methods indicated and held at a temperature of about -21° C. for eleven days before being ground. The samples were all ground and handled as in the previous experiment, except that they were all defrosted and held at a temperature of 4-5° C. On the day that they were ground, identical ammonia contents were found for samples E, F, and G while that for H was somewhat lower, that is, 8.5 milligrams per 100 grams and 5.1 milligrams per 100 grams of sample respectively. The progress of decomposition was then followed with the increases in ammonia recorded in Table 8 and Figure 5.

Discussion of Results.

As has been pointed out by previous workers, the decomposition of nitrogenous material, whether it be meat or fish, is no simple process. Although autolysis undoubtedly precedes bacterial decomposition, during much of the spoilage of fish it would be difficult, if not impossible, to say just which comes first, or to attribute the end products specifically to either activity. However, since ammonia appears as one of the end products of the protein decomposition, regardless of the intermediate steps, and increases at a fairly regular rate, it seems to be a suitable index of the rate of decomposition.

In this connection it should be mentioned that there was a very definite correlation between the amount of ammonia present and the state of preservation of the sample as determined organoleptically. With an ammonia content up to 35 milligrams per 100 grams of sample the fish would have been acceptable as human food to the most exacting individual. In most cases, however, when

the ammonia content had increased to from 35 to 45 milligrams the freshness of the sample would have been questioned. At 60 milligrams of ammonia per 100 grams of sample there was a "stale" or "fishy" odor which became a distinctly putrifactive odor as 75 milligrams per 100 grams of sample were approached.

A study of Table 7 and Figure 4 will show that the rate of decomposition is materially affected by the temperature at which the ground samples are stored. The advantage of holding fish at a temperature lower than that obtained by ice refrigeration is clearly shown.

Table 8 and Figure 5 show that at a given temperature there is little significant increase in the rate of decomposition of defrosted fish when compared with a similar sample which has not been frozen. Thus although the consumer may not have the facilities in the home for holding fish in the frozen condition after its purchase from the retailer, he is still as well off, as far as actual spoilage is concerned, as if he were to hold "fresh" fish under the same conditions. Before its delivery to the consumer even more can be said in favor of the frozen product. As Lumley (31) has pointed out, crushed ice is an excellent medium for keeping fish fresh during the short period necessary for transportation from the near by fishing grounds to a seacoast town. However, since the maximum time that fish can be held in ice and be considered to be really fresh is probably from twelve to fourteen days, the distance that it can be shipped inland is extremely limited. This is especially true in view of the present conditions encountered in the haddock industry. At one time, good fishing could be found relatively near the New England shore. The boats left early in

the morning and were often back with the catch the same day. Now, during much of the year, it is necessary to fish in more distant waters, with the boats out of port from eight to twelve days. Thus the time left for shipping the fish from the coast to an inland consumer, in a really fresh condition, is even more limited than formerly. On the otherhand, however, if as is now done, first quality fish is taken immediately upon the arrival of the boat, filleted, and frozen, the time available for shipment to distant consumers becomes almost unlimited.

It may be important to note that, while samples E, F, and G of Table 8 showed ammonia formation at almost the same rates, sample H was considerably slower. The explanation of this may be found in the bacteriological work of Hunter (23), Fellers (13), and Griffiths and Fuller (16) all of whom showed that while fish flesh was practically always sterile, when caught, the slime on the skin and in the gills was not. Thus, while the commercial freezing of packaged fillets has reached a high degree of success, this may be even further advanced by more careful handling of the fish, before freezing, to prevent the introduction of bacteria from the skin into the flesh of the fish. Any step in this direction may be considered to be a step in the delay of spoilage after defrosting.

In order to check, more accurately, the rate of decomposition of the muscle tissue during the critical period from six to ten days after defrosting, analyses were made as shown in Table 9 and Figure 6. The methods and procedures were exactly the same as those used in obtaining the data of Table 8.

Samples B and S were haddock fillets taken from the same catch and frozen at the Boston Fish Pier by the Birdseye and Sharp methods

TABLE 9

Days held after Grinding	Milligrams NH_3 per 100 Grams of Sample. (B)	(V)	(S)
0	7.34	5.72	5.12
6	8.02	8.02	----
7	5.72	----	8.02
8	5.63	4.01	12.89
9	17.75	11.26	-----
10	25.77	16.13	43.44
11	30.55	17.66	38.66

(B) Birdseye Fillets Frozen at Boston Fish Pier.

(S) Sharp Frozen Fillets from same catch.

(V) Birdseye Fillets purchases from local retailer.

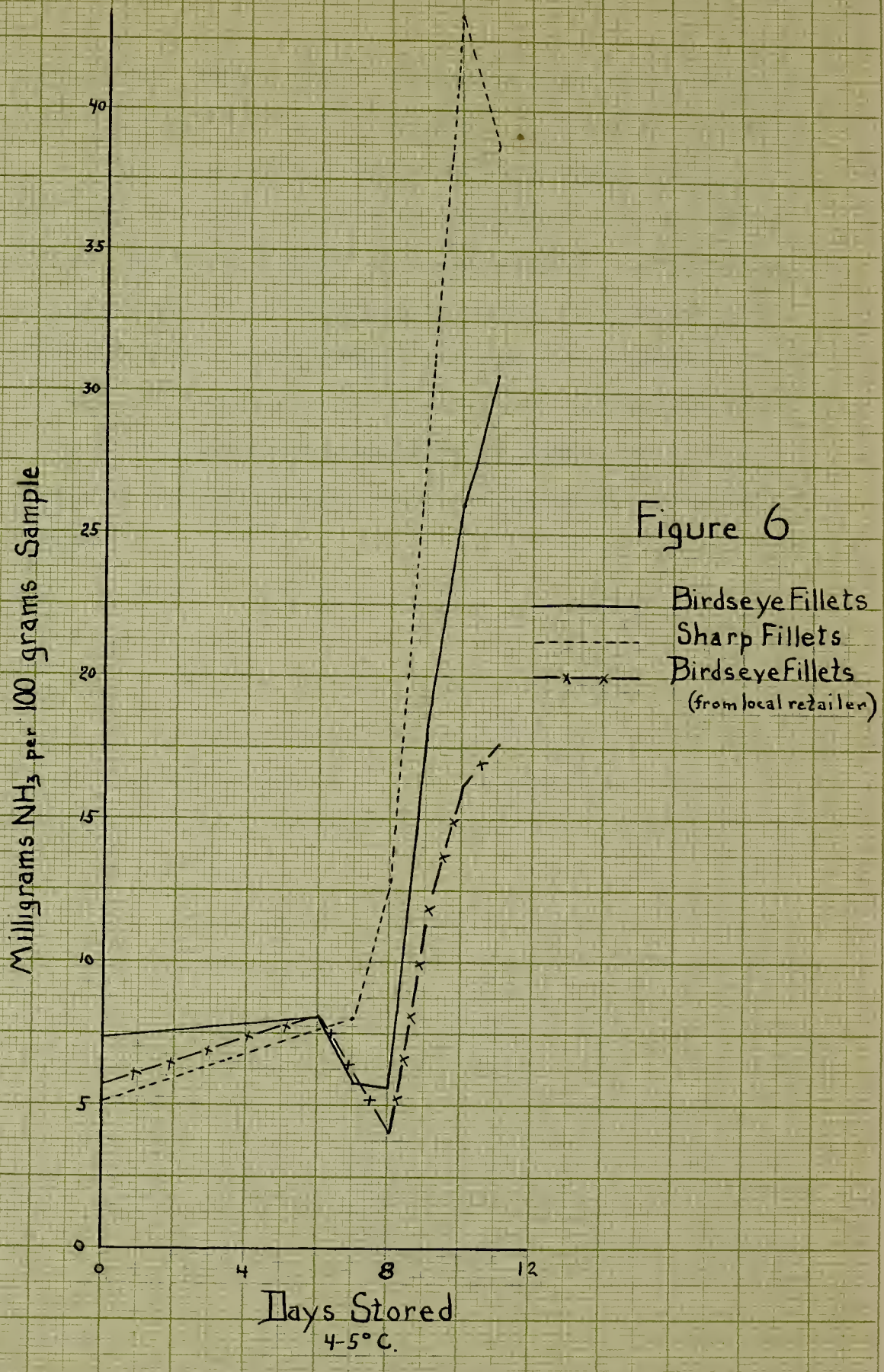

Milligrams NH_3 per 100 grams Sample
0
5
10
15
20
25
30
35
40
0
4
8
12
Days Stored
4-5° C.
Birdseye Fillets
Sharp Fillets
Birdseye Fillets
(from local retailer)

Figure 6

respectively. Sample V was a commercial Birdseye sample purchased from a local retailer after he had held it in stock for some time.

It will be seen from Table 9 that there is practically no ammonia liberated up to six days after defrosting. However, between six and ten days the increase in ammonia formation is both regular and more rapid. It is evident, however, that there is some variation in the rate of decomposition of samples from different catches, but that with samples from the same catch and similarly processed, the rate of decomposition is slower in the samples frozen more rapidly by the Birdseye method than in those frozen by the slower method.

AMINO NITROGEN.

In view of the work previously cited (43) (46) (76) on the amino acids of fish muscle the study of these substances has been limited, in this work, to a comparison of the total quantities found in commercial fillets frozen by the Birdseye and the Sharp methods, both directly after freezing and after storage for one year.

For these determinations twenty-five grams of finely ground muscle, free of bones and skin, were exhausted with 2, 50 milliliter portions followed by 4, 25 milliliter portions of cold, distilled water, then filtered and made up to a volume of 250 milliliters. Using 10 milliliters of this filtrate the amino acid nitrogen was determined, following essentially the proceedure prescribed by the Association of Official Agricultural Chemists (39). Due to the very small amount of amino nitrogen present in the samples, a special micro gas burette was used on a regular macro Van Slyke apparatus.

From the figures given in Table 10 it will be seen that there was no significant difference in the amount of amino acid nitrogen present either when comparing the two types of fillets when just frozen or after storage. In the light of the work by Tressler (76) previously cited, these figures can be interpreted to mean that the samples frozen by both methods had remained in good condition with no appreciable breaking down of the proteins to amino acids, even when stored for a whole year at -5 to -10 degrees Fahrenheit.

TABLE 10

AMINO ACID NITROGEN.

Amino acid nitrogen.

Milligrams Nitrogen per Gram Fresh Muscle.

	One Week After Freezing.	One year After Freezing.
Birdseye Fillets	1.758	1.939
	1.453	1.981
Average	1.6205	1.960
Sharp Fillets	1.956	1.996
	1.956	1.925
Average	1.956	1.9605

SODIUM CHLORIDE SOLUBLE NITROGEN FRACTIONS.

In 1933 Reay (57) working at the Torrey Research Station in Aberdeen, Scotland, observed among other things that, on storage, there was a decrease in the amount of protein material which could be extracted from fish tissue as the length of storage was increased.

In order to study, to some extent, the nature of this change, it seemed advisable to determine the amount of certain nitrogen fractions present in both Birdseye and Sharp frozen fillets, immediately after freezing and also after storage for one year at a temperature of -5 to -10 degrees Fahrenheit.

After considerable preliminary work on various samples, the following procedure was developed. Working on the theory that proteins as a group are soluble in dilute neutral salt solutions, fifty grams of freshly ground muscle were placed in a glass stoppered bottle with about two hundred milliliters of 10 per cent sodium chloride solution and allowed to stand over night at a temperature slightly above 0 degrees Centigrade. The next morning the supernatant liquid was poured off and centrifuged to remove any suspended matter. The residue was repeatedly extracted with small amounts of the sodium chloride solution until the total volume approached five hundred milliliters. This was then made up to a volume of 500 milliliters, using 10 per cent sodium chloride solution, and saved for analysis, while the residue was introduced directly into a five-hundred milliliter Kjeldahl flask in order to determine the total non-extractable nitrogen, following the procedure prescribed by the Association of Official Agricultural Chemists, (42).

The first determination on the extract was that for the total extracted nitrogen, that is, the total nitrogen soluble in a 10 per cent sodium chloride solution.. For this a 50 milliliter aliquot was withdrawn and introduced directly into a five-hundred milliliter Kjeldahl flask, followed by the usual Kjeldahl procedure. (42)

In order to determine the amount of globulin in the extract a 100 milliliter aliquot was measured into a beaker and solid sodium chloride was added until saturation had been reached. The percipitate was removed by filtration and washed with cold saturated sodium chloride solution. It was then washed from the filter into a beaker with a small amount of the saturated sodium chloride solution and boiled for five minutes. The resulting coagulum was collected on a filter, washed with hot water and introduced with the paper into a Kjeldahl flask for the determination of nitrogen.

The albumin was determined in the combined sodium chloride filtrate and washings from the globulin percipitation. These were boiled for 5 minutes with 1 gram of tri-chloracetic acid, filtered, and the percipitate washed with hot water. The percipitate and filter paper were then introduced into a Kjeldahl flask for the determination of the nitrogen as in the previous case.

In order to check the total protein content of the extract, as well as to make possible a more exact calculation of the non-protein nitrogen of the solution, the total coagulable nitrogen was determined. For this, 100 milliliters of the original extract were boiled for five minutes with 1 gram of tri-chloracetic acid. The percipitate was removed by filtration, washed with hot water and the total nitrogen determined as before.

A 50 milliliter aliquot of this last filtrate was introduced into a Kjeldahl flask and the non-coagulable or non-protein nitrogen determined.

From these determinations the following fractions were obtained:

A. Total nitrogen of sample on a fresh basis, calculated from the total nitrogen previously determined on a vacuum dried sample.

B. Total nitrogen soluble in ten per cent sodium chloride solution.

C. Globulin nitrogen, percipitated by saturated sodium chloride

and determined as such.

D. Albumin nitrogen, percipitated by heat and tri-chloracetic acid and determined as such.

E. Total coagulable nitrogen percipitated by heat and tri-chloracetic acid and determined as such.

F. Non-protein nitrogen, determined by direct measurement after removal of coagulable proteins.

G. Non-extractable nitrogen determined by direct analysis.

H. B, plus G, should give total nitrogen by calculation.

From the results of this study as shown in Table 11 it may be seen that on storage the amount of nitrogen soluble in ten per cent sodium chloride decreased considerably during the course of the year. In this work, even more than in other parts of the study, the difficulty of obtaining uniform samples was experienced. Further, the difficulty of separating the fish material from the extracting liquid (10 per cent NaCl solution) presented problems not yet satisfactorily solved. However, this phase of the work does conclusively show that there was a decrease in the amount of extractable nitrogen in the fish muscle during storage, with the loss shared by both the albumin and the globulin.

TABLE 11

NITROGEN FRACTIONS BASED ON SOLUBILITY IN TEN PERCENT SODIUM CHLORIDE SOLUTION.

(Milligrams nitrogen per gram fresh muscle.)

Sample	(A) Total N. Fresh Sample	(B) Total Soluble	(C) Globulin (Satd.NaCl)	(D) Albumin.	(E) Total Coagulable	(F) N.P.N.	(G) Non-Extract	(H) B+G (3)
D^{10} (1)	26.79	10.91	3.53	3.23	7.55	3.44	---	---
D^{10} (2)	26.79	11.42	4.01	3.35	6.80	4.68	14.18	25.60
E^{10} (1)	28.04	11.73	4.38	3.26	8.09	4.39	-----	-----
E^{10} (2)	28.04	12.81	4.69	3.96	7.25	5.31	15.37	28.18
D^{1} (4)	31.15	9.73	3.04	2.41	4.77	3.76	18.93	28.66
D^{1} (4)	31.15	9.49	2.35	3.19	6.18	3.26	19.10	28.59
E^{1} (4)	32.60	10.43	3.21	2.67	5.88	4.10	17.29	27.72
E^{1} (4)	32.60	8.76	1.58	5.15	5.70	2.95	19.57	28.33

(1) Samples allowed to stand over night with 10% NaCl Solution.

(2) Samples allowed to stand two days with 10% NaCl Solution.

(3) Values too low due to difficulty in digesting crude non-extracted mass.

(4) Determinations made after storage for one year.

INORGANIC CONSTITUENTS.*

Due to the rather extensive work which has been done by previous workers on the inorganic constituents of fish in general, and since there is no reason to believe that these constituents would be affected by freezing or storage, the work along that line has been limited, in this present study, to the analysis of the dried muscle for copper, iron, manganese, and phosphorus.

For the inorganic analyses ten grams of vacuum dried muscle tissue were introduced into a five-hundred milliliter Kjeldahl flask to which were added twenty-five milliliters of concentrated sulphuric acid. During the course of the combustion which took about two hours, seventy-five milliliters of concentrated nitric acid were added from a dropping funnel. This gave a product which could be diluted with about three-hundred milliliters of redistilled water and filtered directly into five hundred milliliter volumetric flasks. The Kjeldahl flasks and filters were then washed with several small portions of redistilled water until the filtrate approached a total volume of fi e-hundred milliliters. The funnels were then removed and the filtrates made up to volume at twenty-five degrees Centigrade. These samples were then thoroughly mixed and set aside for analysis.

* In these analyses the methods used were developed in this laboratory by Dr. Edward B. Holland and Mr. R. A. Caughey and based on the earlier work of numerous workers, all of whom will be mentioned in connection with the individual methods.

As in the previous work discussed in this thesis, four sets of samples were compared in each case and identified as before; namely, B, fish frozen at sea by means of solid carbon dioxide as soon as caught and gutted; C, fish frozen whole as soon as landed at the Boston Fish Pier but otherwise handled with the rest of the commercial catch; D, commercial fillets frozen by the Birdseye method ; and E, commercial fillets frozen by the Sharp or so-called "slow" method. Samples of each of these were taken at four different periods in order to roughly devide the data with respect to the four seasons of the year.

COPPER

The copper analysis as developed, based on the earlier work of Callan and Henderson (6), Elvehjem and Lindow (12), McFarlan (35), Mosely, Rohwer, and Moore (37), and Sylvester and Lampitt (70), was carried out on 100 milliliters of the filtrate mentioned above. This was evaporated to dryness with 10 milliliters of concentrated nitric acid. The sides of the beaker were then washed down with redistilled water, followed by 5 milliliters of ammonium hydroxide and 10 milliliters of a 10 per cent solution of citric acid. Ammonium hydroxide was then added drop by drop until the solution was just alkaline to litmus.

The entire solution was then transferred to a 100 milliliter glass stoppered graduated cylinder and washed to a volume of 85 milliliters with redistilled water. Exactly 5 milliliters of a 0.2 per cent solution of sodium diethyl-dithiocarbamate and 10 milliliters of reagent grade carbon tetrachloride were then added. After the stoppered cylinder had been shaken for 2 minutes the mixture was

TABLE 12

Per cent Copper in Dry Haddock Muscle.

Date of Sample	Series B	Series C	Series D	Series E
Aug.20,1935	.00278	.00072	.00064	.00106
Oct.24,1935	.00256	.00141	.00098	.00124
Dec.22,1935	.00084	.00087	.00106	.00101
March.12,1936	.00066	.00075	.00073	.00075
Average	.00171	.00094	.00085	.00102

poured into a sepratory funnel. The layer of carbon tetrachloride containing the colored copper salt was drawn off and run through a small filter paper to remove the last traces of water. This was compared in a Duboscq colorimeter against a standard copper sulphate solution containing 0.00001 grams of copper per milliliter and which had been treated exactly as had the sample filtrates.

The results of these analyses are shown in Table 12. These averages compare very favorably with analyses reported by Lindow, Elvehjem, and Peterson (28) in which they give a copper content of 0.00134 per cent calculated on a dry basis. Parks and Rose (49), when making a study of the copper, iron and manganese content of certain Lake Champlain fishes, analyzed a few haddock in order to compare fresh and salt water fishes. They reported 2.3 milligrams of copper per kilogram of fresh fish muscle. This on a basis of the moisture content which they reported as 79.1 per cent, for the same samples, may be calculated to give a copper content of 0.00110% on a dry basis.

In neither of these reports was any mention made as to the season of the year during which the fish were caught. A study of Table 12, will show that there is a seasonal variation, with a maximum amount of copper found in the muscle during the winter months, and a minimum after the spawning migration during February or early March.

Iron.

Analyses for iron were made on the same ash filtrates that were used for copper, following a method developed in this laboratory based on the earlier work of Kennedy (24), Peterson and Elvehjem (50), and McFarlane (34). It is based on the long recognized reaction between

TABLE 13

Per cent of Iron in Dry Haddock Muscle.

Date of Sample	Series B	Series C	Series D	Series E
Aug.20,1935	.00873	.00502	.00413	.00630
Oct.24,1935	.00390	.00351	.00415	.00368
Dec.22,1935	.00310	.00270	.00280	.00358
Mar.12,1936	.00209	.00198	.00186	.00175
Average	.00446	.00330	.00324	.00383

the ferric iron and soluble thiocyanate, giving the characteristic color of ferric thiocyanate.

Twenty-five milliliters of the original ash solution were measured into a 50 milliliter volumetric flask. To this were added 1 milliliter of concentrated nitric acid and 10 milliliters of an aqueous solution of potassium thiocyanate. (200g/1000 ml.) This was then made up to volume, thoroughly mixed, and compared with a standard iron solution containing 0.0001 grams of iron per milliliter of solution and treated exactly as were the sample filtrates.

The results of these analyses are shown in Table 13. As in the previous work on copper there is no appreciable difference in the iron content of the samples frozen by the various methods. All samples show more iron than was reported by Peterson and Elvehjem (50) and Parks and Rose, (49). The former workers reported only 0.00042 per cent while the latter reported 4.8 milligrams of iron per kilogram of fresh muscle. Again calculating on the basis of their reported 79.1 per cent of water, this figure can be calculated to give 0.00230 per cent of iron on a dry basis.

There is evidence of a seasonal variation which is considerably greater than any difference observed between samples of the same catch but frozen by a different method. It will be noted that the high and low iron analyses, although they do not coincide absolutely with those of the copper, do follow very closely the season of the year.

MANGANESE

The determination of manganese in vacuum dried haddock muscle followed a method based on the work of Reiman and Minot (61). Willard and Greathouse (81), Richards (62), Lindow and Peterson (29), Skin-

ner and Peterson (64), and Bolin (5).

One hundred milliliters of the original ash solution were evaporated to dryness on a steam bath with 5 milliliters of concentrated nitric acid. This was then taken up in 10 milliliters of concentrated phosphoric acid. After the addition of 0.5 grams of potassium periodate, the solution was heated on the boiling water bath for from five to ten minutes, during which time a maximum intensity fo color developed.

In this work the extremely small amount of manganese present did not give color enough to make a color comparison against a standard possible. A distinct difference was noted with a maximum color produced in the samples of October 24th, decreasing through December to give a minimum in March, followed by a slight increase in August. Using a very similar method, Lindow and Peterson (29) reported no manganese in Halibut or Blue fish, while Peterson and Skinner (51) reported manganese contents of 0.0063 per cent for Cod and Red Snapper. Very little work seems to have been done on the manganese content of fish muscle and no perfectly agreeing data have been found for the manganese in Haddock.

From this present observation of the variation in the manganese content with the season, it may be concluded that although the Haddock is not considered a migratory fish in the sense that the Salmon is, there is a very difinite storage of minerals in the muscle of the haddock in preparation for the spawning season.

Phosphorus.

Phosphorus was determined in the same four representative samples, using essentially the method of Fiske and Subbarow (16). It was ap-

TABLE 14

Per cent of Phosphorus in Dry Haddock Muscle.

Date of Sample	Series B	Series C	Series D	Series E
Aug.20,1935	1.1267	0.9736	1.0000	0.9412
Oct.24,1935	1.1429	1.1726	0.9413	0.9303
Dec.22,1935	1.0656	1.0666	1.0000	1.0204
Mar.12,1936	1.0423	1.0000	0.9581	0.9877
Average	1.0946	1.0545	0.9749	0.9499

plied to the present problem as follows; ten milliliters of the original ash filtrate were introduced into a one hundred milliliter volumetric flask, and diluted to about seventy milliliters. To this were then added 10 milliliters of ammonium molybdate solution and 4 milliliters of a 0.25 per cent solution of amino-naphthol-sulphonic acid. A maximum color developed in about seven to ten minutes at which time the sample was compared with a standard solution of monopotassium phosphate containing 0.00008 grams of phosphorus per milliliter of solution.

Table 14 shows that there was no significant variation in the phosphorus content either among the samples frozen by the various methods or among samples taken at different seasons of the year.

EXPERIMENTAL PART II

NUTRITIONAL STUDIES

As has been pointed out earlier in this paper, most of the experimental work on the nutritive value of fish has been done by calculating the theoretical food value, from chemical analyses, and very little has been published to date in which the relative nutritive values of frozen fish have been determined biologically.

Therefore it was proposed to study the nutritive value of fish muscle, frozen by the four methods previously described, that is, frozen at sea by means of "dry ice" as soon as caught and gutted, frozen whole at the Boston Fish Pier by the Birdseye method but otherwise handled as the rest of the commercial catch, and fillets frozen by both the Birdseye and the Sharp methods. As in the chemical work these four samples will be referred to as Series B, C, D, and E respectively. To do this it was proposed to use the various types of frozen fish as the sole source of protein in a synthetic diet for white rats. From data covering the growth, reproduction, and lactation it seemed that rather conclusive results might be obtained, especially in view of the plan to carry the rats through three generations.

In order to carry out this plan, six series of animals were started, each consisting of three young females and an unrelated male of Wistar stock which had been bred in this laboratory for several generations. Two of these series will be referred to as "Series A" and "Series F." These were carried as controls through the first generation. After the first generation "Series A" was the only con-

trol carried. The control animals were carried on a diet of Purina Fox Chow. Although this was fed adlibitum observations made over a period of several weeks showed that an average animal during the growing period ate approximately nine grams of the dry feed per day. This figure must be taken as only approximate because some animals ate more than others, while some wasted much more. Since over a period of time this figure seemed to be a rather acurate average, it will be used in making certain future calculations.

The other four series of rats, hereafter referred to as "Series B, C, D, and E" were fed diets consisting of 60 parts by weight of freshly ground fish muscle, 30 parts of dextrinized starch, 5 parts dried brewers yeast, 5 parts of salt mixture (Osborne and Mendel) (47) 6 parts of butter fat, and 2 parts of cod liver oil. In each case the fish muscle used corresponded to the sample B, C, D, and E discussed under the chemical part of this study.

This diet was fed ad libitum and the intake was followed over a period of several weeks. It was found that an average of twenty-two grams per day was eaten by the growing rats.

Analyses of the mixed diets and Fox Chow were made and it was found that when the food value and composition were considered, the control and test animals were receiving a remarkably similar diet. It should be borne in mind, however, that there was probably slightly less waste of the synthetic diet because of its consistency. However, the actual protein intake (N x 6.25) was very similar in amount for both the animals receiving the fish muscle as their sole source of protein and the control animals whose protein, according to the manufacturers of the Fox Chow, was of both animal and vegetable origin.

This experimental diet was similar in composition to that used by Nelson (38) in studying the effect of beef as the sole source of protein. She used, however, 50 parts of beef, 30 of starch, 5 of yeast, 5 of salt, 8 of fat, and 2 of cod liver oil, with an additional 1 part of sodium chloride. In order to keep the calorific intake of the animals on the synthetic diet as near as possible to that of the control animals only six parts of butter fat were used in this work.

For the first generation eighteen females and six males of good healthy stock and about six weeks old were taken from the stock colony. These were separated into six cages of three females each while the males were kept in individual cages until they were 110 to 115 days old, when they were put into the corresponding cages with the females on the same diet.

All animals were weighed once a week and from the average net increase in each case the results of the six series were plotted as shown in Figure 7. From this it will be seen that there was practically no difference in the rates of growth until between five and six weeks had elapsed. At that time the animals on diets B, C, and E started to gain more rapidly than did the control animals. During the eighth week those on diet D also surpassed the controls in weight with the result that after having been on the five diets respectively for ten weeks, those on the synthetic diets with fish as the sole source of protein had in every case surpassed those on the supposedly complete diet as represented by the Fox Chow.

In recording the growth of the second generation of animals on the same four fish diets and the control, the use of a larger number of animals, and the fact that they were not all in the same stage of

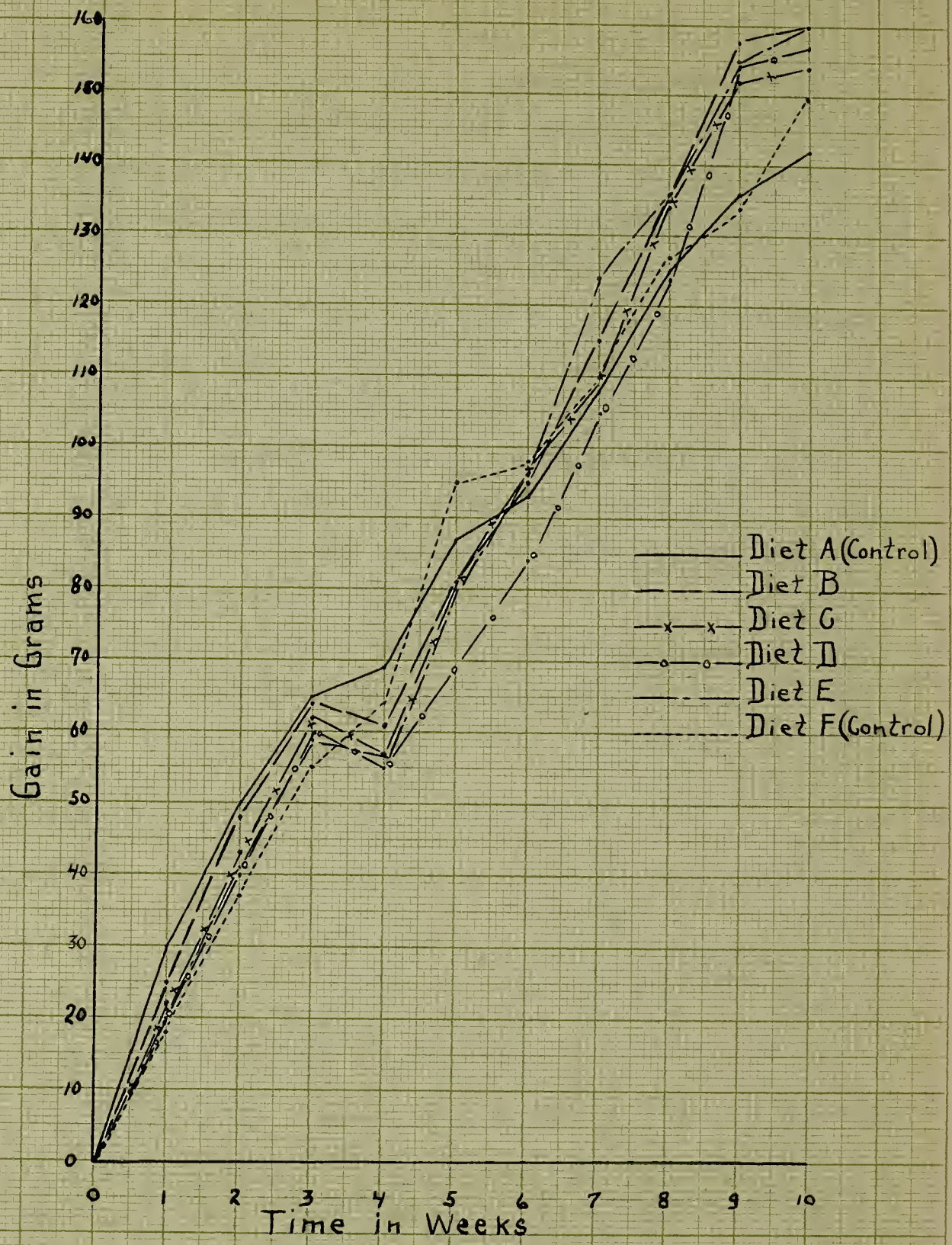

Gain in Grams
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
Time in Weeks
Diet A (Control)
Diet B
Diet C
Diet D
Diet E
Diet F (Control)

Figure 7

development at the same time, made some other means of comparison seem advisable. Therefore, in order to compare the growth of the animals of the second generation, all animals were weighed weekly, as before, from the time they were weaned (21 days) until they reached the age of 120 days. A summary showing the averages for these weights is given in Table 15, and is graphically represented in Figure 8. This summary gives the data compiled from tables representing fifty-three animals followed through the growing period. As will be seen both the males and females on diet D (Birdseye Fillets) made the greatest gain, while the poorest (by males) was made on diet B. (Fish frozen whole at sea). The females making the poorest gain were those on the control diet.

Figures 10, 11, 12, and 13 show animals of the second generation at maturity, comparing control and fish diets.

TABLE 15

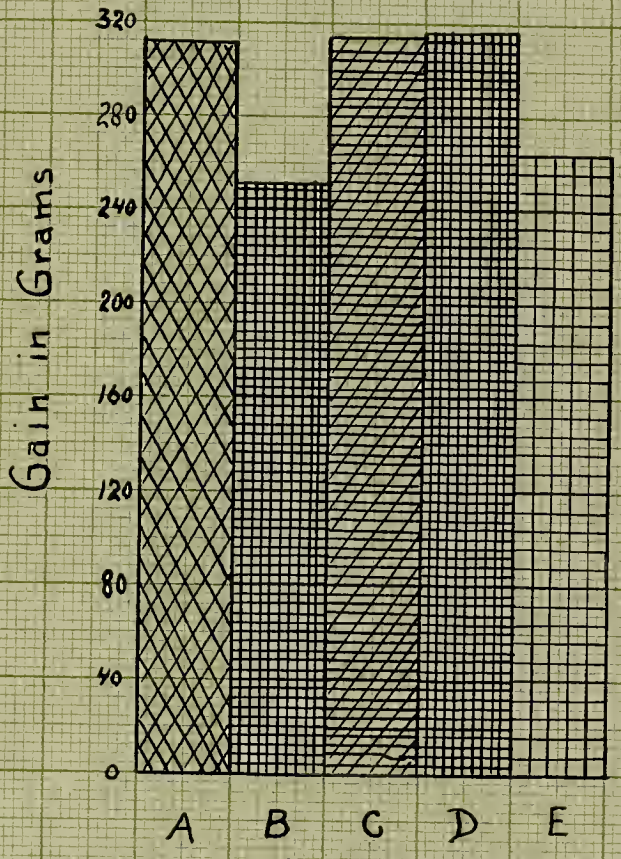
SUMMARY OF GROWTH? SECOND GENERATION.

MALES

Series	Aver. weight 21 days.	Aver. weight 120 days.	Average Gain. (grams)
A	45.0	357.5	312.5
B	39.8	290.7	250.9
C	36.0	350.0	314.0
D	42.9	359.6	316.7
E	33.9	297.9	264.0

FEMALES

Series	Aver. weight 21 days.	Aver. weight 120 days.	Average Gain. (grams)
A	29.0	190.8	161.8
B	36.0	207.8	171.8
C	40.5	207.0	166.5
D	39.6	229.1	189.5
E	32.0	196.0	164.0



A. Control Diet

B. Fish Frozen by CO_2

C Fish Frozen whole by Birdseye.

D Birdseye Fillets

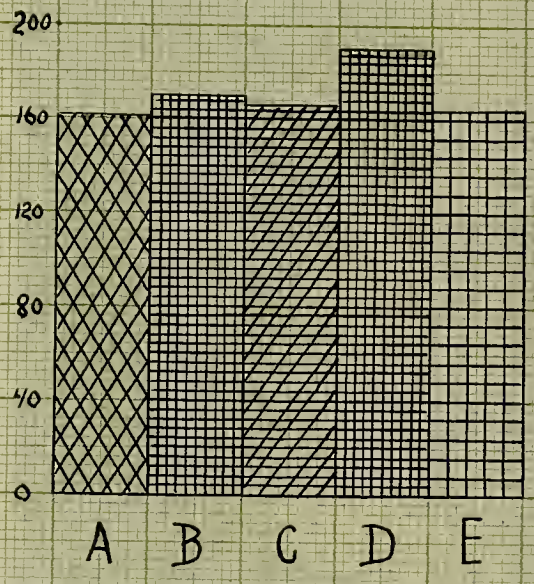
E Sharp Fillets.

Figure 8

Growth.

2nd. Generation.

Males



A. Control Diet

B. Fish Frozen by CO_2

C. Fish Frozen Round by Birdseye

D. Birdseye Fillets

E. Sharp Fillets

Figure 8A

Growth

2nd Generation

Females

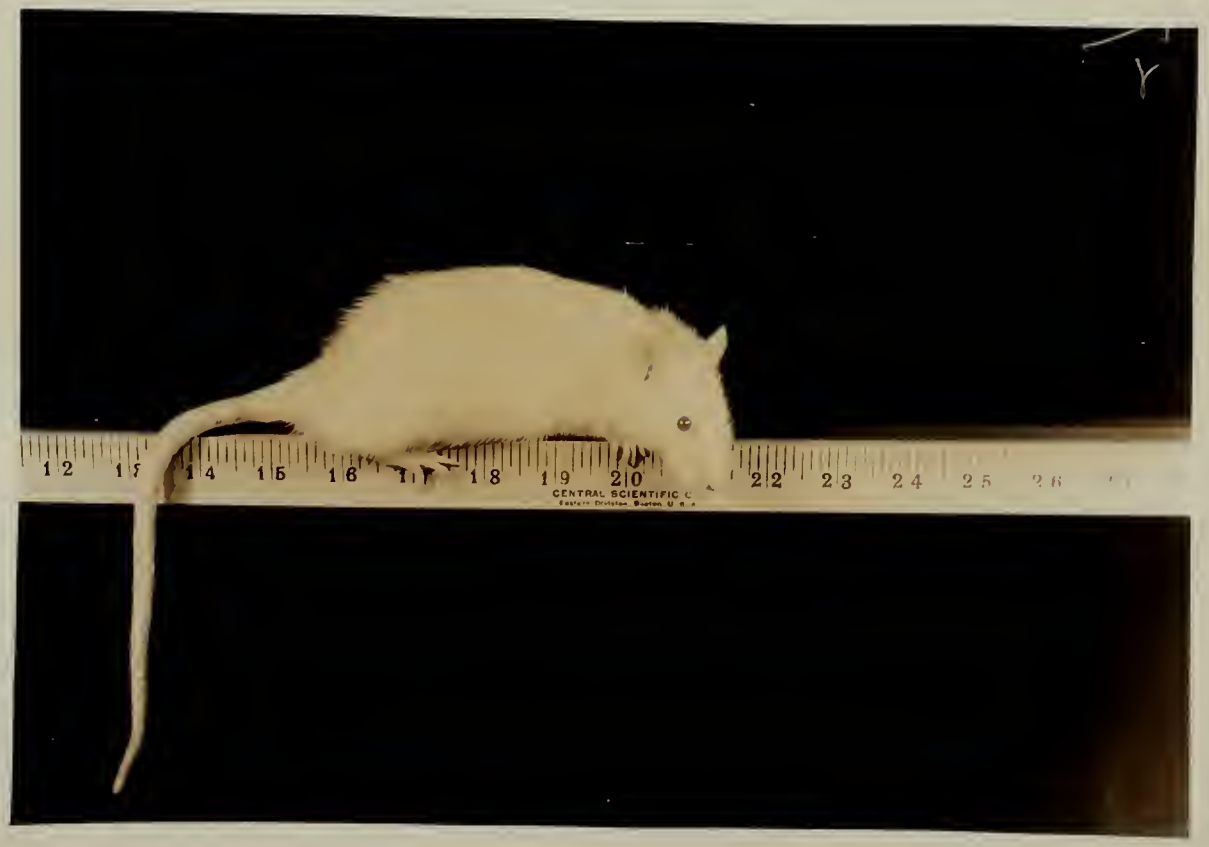


Figure 10

Female #27 Series A

Weight 190 Grams at 120 days.

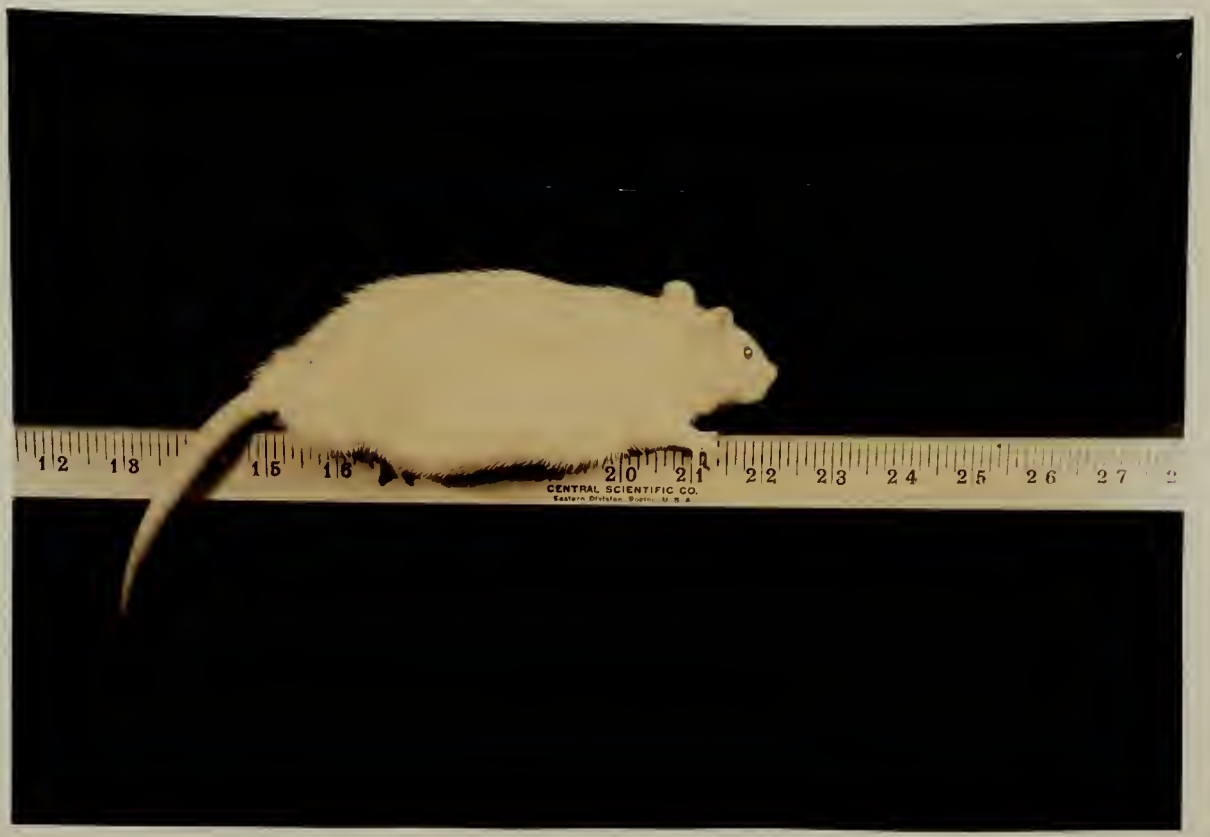


Figure 11

Female #5 Series D

Weight 252 Grams at 120 days.

Figure 12

Male #12 Series F.

Weight 276 Grams at 120 days.

Figure 13

Male # 59 Series D.

Weight 353 Grams at 120 days.

REPRODUCTION AND LACTATION.

In order to further study the value of fish muscle as a source of complete protein rather extensive records were made covering the reproduction and lactation of the same animals included in the report of the growth of the first generation. From the first litters born of these animals, a second generation of breeders was started, using the same diets as had been used for the parent generation.

Rather than burden this thesis with the complete records of these data, summaries are given. Table 16 shows the total number of young born and weaned, as well as the per cent of those born which lived to the weaning age of twenty-one days. The same table also shows the average weight of the young at the time of weaning. Although this latter figure must be influenced to a certain extent by the size of the litters, it is also an indication of the milk available for the young. The same data are shown graphically in Figure 9.

From these data it will be seen that although the animals on the control diet weaned a slightly larger per cent of the young born, the average weaning weight was less than on any of the fish diets. On the other hand, the animals fed the diet in which the Birdseye fillets were the sole source of protein, not only weaned nearly as large a per cent of their young, but the young at weaning averaged 5.7 grams heavier than their nearest rivals, namely, those fed on the diet containing the fish frozen whole at sea. The per cent of young weaned on the B diet, however, was only 35 per cent as compared with 55.7 for those on the D or Birdseye Fillet diet.

Of more interest to the commercial freezer of fish is a comparison between the diets composed of Commercial "Quick" and Commercial

"Slow" frozen fillets. (D and E) While the animals on the former weaned 55.7 per cent of the young born with an average weaning weight of 42.9 grams, the animals on the latter (E) diet weaned only 26 per cent of the young born and with an average weaning weight of only 33.4 grams.

An attempt to carry this work into a third generation was extremely discouraging. Table 17 shows the data for the second generation breeders, covering reproduction and lactation. Although a few litters were born none were really successful. The one conclusion that can be drawn from a comparison of these tables is that frozen fish, especially that frozen rapidly, is an extremely valuable source of protein for growth, reproduction and lactation through the first generation but, after that, reproduction and lactation require some additional food factors not supplied by the fish. The time available for this study was not sufficient to determine the nature of this deficiency.

The difficulty in carrying animals beyond the first generation was also encountered by Smith and Seegers (66) when studying beef liver as a sole source of protein. In the cases of both the liver and haddock diets, the problem seemed to be one of faulty lactation.

TABLE 16

REPRODUCTION AND LACTATION - FIRST GENERATION.

Series	Total Number Born.	Total Number Weaned.	Percent Weaned.	Aver. Weaning Weight. (grams)
A	53	31	58.5	31.1
B	40	14	35.0	37.2
C	70	5	7.1	35.4
D	106	59	55.7	42.9
E	73	19	26.0	33.4
F	103	66	64.1	31.7

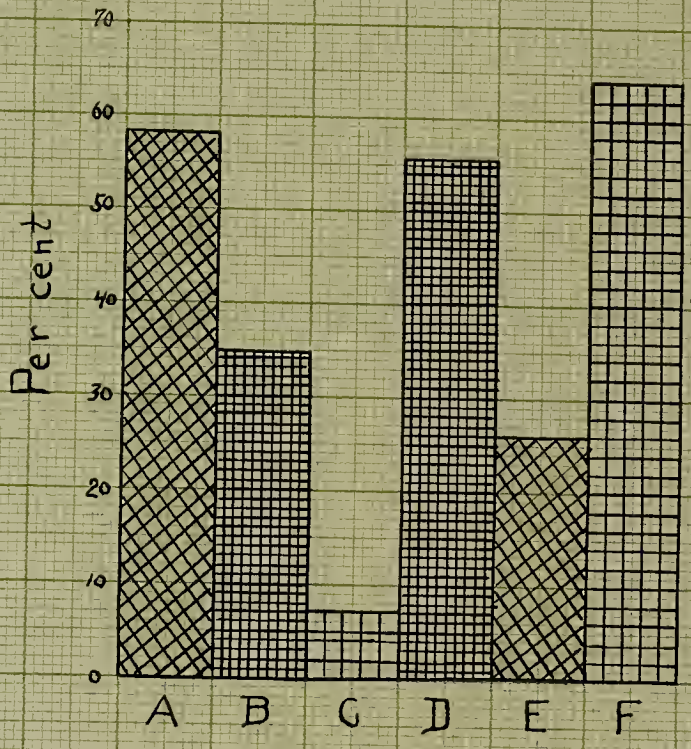


Figure 9

Per cent Weaned
by
First Generation Breeders

A. Control Diet
B. Fish Frozen Round by CO_2
C. Fish Frozen Round by Birdseye
D. Birdseye Fillets
E. Sharp Fillets.
F Control Diet

TABLE 17

REPRODUCTION AND LACTATION - SECOND GENERATION.

Series	Total Number Born.	Total Number Weaned.	Per cent Weaned.	Aver. Weaning Weight. (Grams)
A-F	64	34	53.1	30.4
B	45	2	4.4	31.5
C	32	1	3.1	45.0
D	15	0	0.0	----
E	17	0	0.0	-----

VITAMINS A AND D

As has been pointed out, considerable work has been done by numerous workers (55) in studying the vitamin content of various fish oils. In view of the low fat content of haddock muscle one would normally expect to find very little of the fat soluble vitamins in the muscle tissue. However, to prove this point assays were made for both vitamins A and D, following the methods prescribed by the 1934 Interim Revision, Announcement #3, of the United States Pharmacopoeia (80), with some rather interesting results.

Attempts to assay fish muscle from Series B and C (fish frozen whole) for either vitamin A or D were failures because in both cases the depleted animals absolutely refused to eat the supplements. The animals would refuse to eat fresh fish supplements in their cages, until eventually they either died with all the symptoms of a vitamin A deficiency or, in the case of the animals testing for D, became so crippled that they were chloreformed.

The animals fed the fillets, frozen by either the "Quick" or "Slow" methods, however, in most cases ate them readily, when supplements were given in daily doses up to and including 10 grams of fresh muscle, per animal.

On this basis, using the value of a twelve-gram gain in body weight during the twenty-eight day test period as being equal to one Sherman or 1.4 International units, fillets frozen by the "Quick" method were found to have an average Vitamin A potency of 0.252 International Units per gram of fresh muscle tissue. Fillets frozen by the "Slow" method were likewise found to contain 0.256 International Units per gram. This could not be interpreted to

mean that one method destroyed the Vitamin A while the other did not because not only was it observed that some animals wasted more of the supplement than did others, but it was pointed out earlier in this work that the fat content of the muscle tissue was not always the same.

Similar results were obtained in the Vitamin D assays. Some of the rats ate the supplements readily while others refused to eat them at all. That is, it was impossible to get them to eat the supplements which had been frozen round while , in most cases, they ate the frozen fillets readily.

Using the McCollum (33) (19) line test, plus-four healing was observed in the case of all animals which ate the supplements of fillets, frozen by either method, in an amount equivalent to 10 grams of fresh muscle tissue per day for the required eight days.

Of further interest is the fact that when similar assays were made on fillets which had been frozen by both methods and stored for a year, plus-four healing was again observed on the animals which ate the ten-gram-per-day supplements of "Quick" frozen fillets. The rats which were given the "Slow" fillets which had been stored for a year either refused to eat them at all, or ate them so irregularly that the results could not be interpreted to mean anything. This refusal to eat the supplements is unexplained, because, as far as could be observed, the fillets frozen by the two methods were in as equally fine condition, even after storage for a full year.

Figures 14 and 15 show two animals, one fed on a Vitamin A-free diet while the other received in addition 10 grams per day of Birdseye haddock fillets.

Figure 14

Male #159 Vitamin A-Free Diet.

Figure 15

Male. #145 A-Free Diet
Supplemented by
10 Grams "Birdseye" Fillets per day.

SUMMARY

Water, ash, ether extract, organic nitrogen, ammonia, copper, iron, manganese, and phosphorus were determined on four series of haddock samples caught over a period of one year. These four series of samples included fish frozen whole at sea as soon as caught, by means of solid carbon dioxide, fish frozen whole by the Birdseye method as soon as landed, and commercial fillets frozen by both the Birdseye and Sharp methods. No significant difference was found in the composition of samples frozen by the various methods, although some seasonal variation was found in certain constituents.

Nitrogen fractions soluble in 10 per cent NaCl solution and amino acid nitrogen were determined on representative samples.

Ammonia was found to be a rather accurate index of the state of preservation of the samples. The rate of decomposition of defrosted fish muscle was found to be considerably affected by the storage temperature and to a lesser degree by the method of freezing.

A synthetic diet in which haddock muscle, frozen by each of the four methods previously mentioned, was the sole source of protein was found to be adequate for the growth and reproduction of white rats through two generations but inadequate for lactation beyond the first generation.

Haddock fillets frozen by either the Birdseye or Sharp methods were found to contain measurable amounts of vitamins A. and D.

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